TRYPANOSOMIASIS.

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Thesis submitted for the M. D. Edin.

by

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M.B. Ch.B. Edin. 1911.

October, 1912.

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Introduction.

In recent years a vast amount of literature has been written dealing with Trypanosomiasis and sleeping sickness, and the great importance of human Trypanosomiasis lies in the frequency of its occurrence and its widespread distribution. In this respect and as a causative factor in producing inefficiency in the tropics, it is of importance second only to Malaria.

The discovery by Castellani in 1902 of a trypanosome in cases of sleeping sickness identical with T. Gambiense, formed the starting point of inquiries which make it certain that the parasite is the causal agent of the condition. Being greatly interested in this subject, I have thought that a brief account of our present knowledge of Trypanosomiasis in man would be suitable for a thesis and accordingly in the present thesis, I will give an account of the present position of our knowledge of the subject and point out the various questions that stand in need of further investigation.

The trypanosomata are protozoal organisms belonging to the sub-class flagellata, and many members of the genus have come to be recognised as living in the blood and tissues in various animals and as causing important disease conditions. As long ago as 1878

the T. Lewisi was observed infesting the blood of rats, though it rarely causes their death. There are many other diseases in animals in which similar organisms have been found though space will only permit of the enumeration of a few of the more important. Every year is adding to their number: Surra which occurs in cattle, horses and camels in India and is associated with the T. Evansi; Dourine, a condition affecting horses in the Mediterranean littoral (T. Equiperdium); Mal de Caderas, a disease of South American horses (T. Equinum); Nagana or Tse-tse Fly Disease affecting horses and herbivora in South Africa (T. Brucei); and most important from the human standpoint - the Trypanosomiasis and sleeping sickness of West and Central Africa associated with the T. Gambiense and T. Ugandense, which are now known to be the same organism. These diseases present many general resemblances to each other. They all tend to be characterised by wasting, cachexia, anaemia, fever usually of an intermittent type and irregular oedemas. In many cases the infective agent is now known to be carried from the diseased to the healthy animal by means of blood-sucking insects. And this is the main fact in the biology of those trypanosomata with which the pathologist is concerned - that in the higher animals the parasite is transferred from one host to another by means of these insects or other similar agencies. It

is now definitely known that the mere mechanical transference of the parasite by these insects, while it may sometimes occur, probably plays a subsidiary part in infection. In many cases it has been shown that an insect does not become actively infective for several days after it has sucked the blood from an infective animal. The analogy of the malarial organisms suggests the occurrence of a sexual conjugation within the insect, though definite proof is still wanting. And, as Minchin points out, while we must admit the existence of a cyclic development, it by no means follows that this includes a definitely sexual stage, although many are of the opinion that such a stage does take place.

The study of Trypanosomes, or at least some of them, presents considerable difficulties. Several mammalian trypanosomes resemble one another so closely that it is impossible to distinguish them by their morphological characters alone. This applies to the trypanosomes of Nagana, Surra, Dourine and sleeping sickness. Moreover, trypanosomes belonging to distinct species may present close analogies from the point of view of their pathogenic action on certain animals. On the other hand, the virulence of a trypanosome is influenced by certain factors. It varies especially with the origin and race of the animals inoculated. A trypanosome only slightly virulent for a species of

animal from the Sudan, for example, may be very virulent for the corresponding European species. Also the same trypanosome may present slightly different morphological characters in the blood of different species of animals. It follows, therefore, that it may often be difficult to identify a trypanosome, and on that account several of these haematozoa still lack identification.

Historical Outline.

In 1803 the English observer, Winterbottom, recorded the occurrence among the negroes of the West Coast of Africa of a peculiar disease characterised particularly by a tendency to sleep. Since that time the disease has been known as sleeping sickness and has found a place in all Treatises on Tropical Medicine.

Little progress was made either clinically or pathologically during the next fifty years. From 1861 to 1900 French Naval surgeons published a series of very interesting papers, that of Suerin deserving special mention. In the course of a dozen years he observed as many as 148 cases.

The conquests of Bacteriology were destined to receive, and have received, their set-back in the history of sleeping sickness. Cagigal, A. de Figueiredo, Lepierre, Marchoux, Brodenand, Castellani have all attributed to different organisms the role of pathogenic agent in sleeping sickness.

Several observers have attributed the disease to bad or insufficient feeding. Ziemann held the opinion that the disease was due, not to an infection, but to an intestinal intoxication. The root of the manioc and mud fish have in turn been labelled as causal agents.

In 1900 Manson gave a very full account of two cases that died in Charing Cross Hospital and the pathological anatomy was very fully worked out by Mott.

Manson attributed sleeping sickness to Filaria perstans but it was shown to be merely an incidental infection. It is found in districts where the disease does not occur and in many cases of sleeping sickness the filaria could not be found. This hypothesis of Manson's had, therefore, to be given up.

The researches of Dutton and Todd, of Castellani, and of Bruce, Nabarro and Greig mark the next phase in the elucidation of the aetiology of sleeping sickness.

Dutton in 1901 recognised Trypanosomes in the blood of a European patient suffering from sharp attacks of fever which were looked on as malarial in origin. He gave an excellent description of this parasite which he called the Trypanosoma Gambiense. His results were rapidly confirmed by these other observers; but the relationship between human Trypanosomiasis and sleeping sickness still remained unsuspected when Castellani, examining the cerebro-spinal fluid of negroes suffering from sleeping sickness, discovered Trypanosomes. This momentous discovery was immediately confirmed by Bruce and Nabarro and the parasite was called the T. Ugandese.

At first Castellani was inclined to look on

the presence of the protozoon as accidental, but Col. Bruce, on going out with Nabarro and Greig in 1903, realised the significance of the observation, urged Castellani to further inquiries, which he continued himself after the departure of the latter, with the result that in a series of examinations carried out in several infected localities, the trypanosome was demonstrated in every case of the disease. This work formed the starting point of further inquiries, the results of which will make it certain that the parasite is the causal agent of the condition.

It still remained to be seen if T. Gambiense and T. Ugandense were different species, or if the parasite found in sleeping sickness patients was really the T. Ugandense.

Bruce, Nabarro and Greig showed that 23 out of 80 natives from endemic districts of Uganda had trypanosomes in their blood, whilst in 117 natives from uninfected areas the blood examination was negative in every case. The same observers have shown that, contrary to the observations of Castellani, there is no appreciable difference between T. Ugandense and T. Gambiense, and Todd, Dutton and Christy in their "Report on Trypanosomiasis on the Congo" arrived at the same conclusion. The latter further state that the parasites as seen in the circulating blood are ident-

ical, and the fact that the pathogenic action of the two trypanosomes upon different species of mammals is the same supports this hypothesis. It is now universally believed that T. Ugandense and T. Gambiense are the same species and according to the rules of the priority of nomenclature the name T. Ugandense should disappear and the older name T. Gambiense be retained.

From this till 1910 T. Gambiense was looked on as the only species which caused human Trypanosomiasis. In that year attention was drawn to the fact that in Northern Rhodesia and Nyasaland sleeping sickness had in several cases, European as well as native, been there contracted, and we know that this is outside the area of distribution of Glossina palpalis. Bagshawe writes as follows : "It has been suggested that Rhodesian disease is not sleeping sickness, i.e. that the trypanosome is not Gambiense. The suggestion seems to me too speculative to merit discussion."

Recent research has, however, thrown a new light on the subject and the modern tendency is to admit two species, distinct but near, and nearer to one another than either is to the other pathogenic species. As I will show in a later section the T. Rhodiense and T. Gambiense present important differences. These may be shortly summed up here — their longer incubation period, their morphological differences, and chiefly by the greater pathogenicity of the T. Rhodiense for the majority of animal species.

Sketch Map of

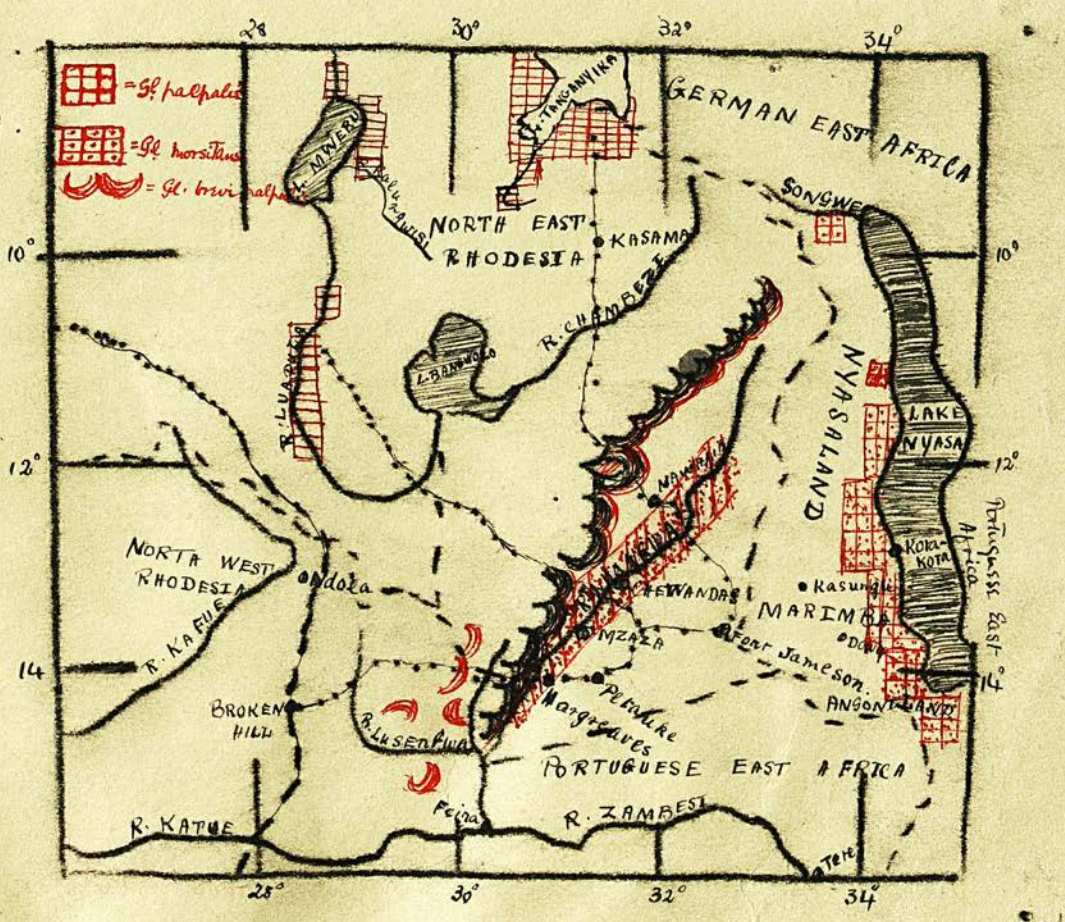
NORTHERN RHODESIA.

Scale 125 miles to 1 inch

Railway Completed

" proposed or under construction

Political Boundary



Sketch map of Southern part of Uganda.
Scale about 55 miles to the inch

——— Red line indicates Sleeping Sickness Areas.

Etiology.

(1) Geographical Distribution.

Trypanosomiasis is endemic only in certain parts of Equatorial Africa, the chief foci of the disease being found along the West Coast from Senegal to St. Paul de Loanda.

In Senegambia the disease is common in Casamance and in Sine and Saloum. In Gambia it is by no means rare; and as we have seen above, it was in Gambia that trypanosomes were first discovered in human blood. Sleeping sickness is prevalent from the mouth of the Gambia to a distance of 250 miles up the river.

Among the most infected areas may be mentioned: Upper Guinea, the hinterland of Sierra Leone, of Liberia, of the Ivory Coast, Lobi and Yatenga. On several occasions the disease has become epidemic at Roba, in Yatenga, from 1886-1889, 300 natives dying from sleeping sickness. The islands of Princes, St. Thomas and Fernando Po are also infected.

In Portugese Congo (Angola) the disease occurs principally in the region of Quissama and along the banks of the River Coanza. In the town of Dondo in 1900 there were 98 deaths out of a population of 2,000 negroes and half-castes. Near the mouth of the Coanza practically the whole population has been carried off by the disease.

Sleeping sickness is endemic in the French Congo and in the Congo Free State, where for several years it has been causing great ravages. The disease is endemic in the neighbourhood of the falls, especially at Banza Manteka. In the training College at Berghe-Ste-Marie the mortality from sleeping sickness increased from 13% in 1896 to 70% in 1900. The majority of the children died of the disease.

The whole population of the left bank of the Congo, from the mouth of the Kasai to Bolobo, higher up the river, has fallen a prey to the scourge which has decimated the villages of Botanquis.

For some years trypanosomiasis had made great ravages in Uganda. The disease appears to have broken out first in Busoga in 1896. Dr. Moffat, then P.M.O., Uganda and East African Protectorates, explains its first appearance there by the fact that a large number of Emin Pasha's Soudanese, with their wives and followers, were brought into and settled in Busoga. They came from the Congo Free State, where sleeping sickness has been endemic for many years. Finding the necessary conditions present, the disease rapidly spread and in a few years became endemic around the Northern shores of the Victoria Nyanza - from Budda on the West to South of Kavkiondo Bay on the East - in Busoga and Chagwe, around Entebbe, and on the islands in the lake. So merciless has been its onslaught that it has claimed

probably more than 200,000 victims in the last ten years.

The distribution of the disease in Uganda is peculiar. Except in the district of Busoga, sleeping sickness has not become endemic far from the shore of the lake or from the banks of rivers. The villages along the lake shore and on the islands of the lake have been almost decimated, many of them having lost two-thirds or more of their inhabitants. The extent to which the disease spreads inland from the lake varies in different parts from ten up to thirty or forty miles. As Bruce, Nabarro and Greig have shown this distribution coincides with, and is probably dependent upon, that of the Tse Tse fly.

According to dates the disease is manifestly spreading in different parts of Uganda, and the Uganda railway has facilitated its spread. It is possible too that the disease may spread to Egypt along the valley of the Nile as far as Glossina Palpalis extends. The dangers of this are not great as this Tse Tse has not been found along the Nile north of Gondokoro; nor is it likely to occur there as the character of the country is unfavourable to the fly. We know, however, that several other species of Tsetse occur in the Anglo-Egyptian Sudan, in British and German East Africa, in British Central Africa, and as far South as Natal

and the Transvaal. If other species of Glossina can act as carriers of T. Gambiense, there is grave danger of sleeping sickness spreading East to the coast, North into the Sudan, and South through British Central Africa even as far as the Transvaal - a danger which the latest reports from Rhodesia only confirm.

We may summarise the information we already possess about the geographical distribution of this disease by saying that it extends along the valleys of the Senegal, Niger, Congo, and Upper Nile, in the valleys of the less important rivers between them, and in Northern Rhodesia.

(2) The Tsetse fly.

It is important to be able to recognise Tsetse flies, especially the glossina palpalis. As I have already shown, mere mechanical transference probably plays a minor part and its infectivity may last over months.

The Tsetse flies are ordinary-looking brownish or greyish-brown flies with a prominent proboscis. The hinder half of the abdomen in most species is of a paler colour and marked by dark brown bands (Glossina Palpalis is an exception to this) which are interrupted in the middle line; the abdomen is, however, invisible when the insect is at rest, as it is then concealed by

the wings. The sexes can easily be distinguished as in the male the external genitalia form a conspicuous knob-like protuberance beneath the end of the abdomen, which is absent in the female.

Only an experienced observer can recognise them when on the wing, but in the resting position their identification is easy. In this position they can be distinguished from all other Diptera, especially the Genera Haematopota and Stomoxys for which they are most likely to be mistaken, by the fact that the wings lie closed flat over one another down the back like the blades of a pair of scissors, while the proboxis (ensheathed in the palpi) projects horizontally in front of the head. As Col. Bruce has pointed out, the closed wings thus give it an elongated appearance. Apart from the prominence and position of the proboxis and the scissor-like position of the wings when at rest, the tsetse fly shows nothing remarkable or striking in its appearance.

As already pointed out, apart from the species of Stomoxys and Haematopota, confusion can hardly take place. The Stomoxys also has a prominent proboscis but as it is not ensheathed in palpi it has a much more slender appearance than that of the Glossina. They are little greyish flies with black markings and are much smaller than tsetse flies, with the exception of

Stomoxys inornata and S. Omega which are almost black and quite often as large as tsetse flies. The wings when in the resting position diverge at an angle like those of the common house-fly and they are thus easily distinguished.

Haematopota, on the other hand, which is a genus of small horse-flies commonly known as clegs, resembles Glossina somewhat closely when at rest. They are of the same size as the larger tsetse flies and of the same brownish colour and elongate shape. In no case, however, is the abdomen marked with dark bands on a light ground, while the wings in the resting position do not close one over the other but diverge slightly at the tips and are also somewhat tectiform, i.e. they meet together at the base like the roof of a house. The antennae, too, afford a further means of distinction. While the antennae of tsetse flies, as of all Muscidae, are drooping, those of Haematopota project horizontally in front of the head and being of some length are readily seen.

The Glossina palpalis possess some distinguishing features which are of value in their further elimination from other species of tsetse-fly. They are rather less than half an inch long (excluding the proboscis and wings); the body is almost black in colour with the exception of a pale patch on the abdomen; the black segments or tarsi of the hindermost pair of legs

are characteristic. Those familiar with G. palpalis at once recognise it by its dark coloration.

There are seven other species of Glossina, the differentiating features being mainly those of colour and of size.

Reproduction.

The tsetse flies do not lay eggs. One larva at a time develops in the mother-fly. This, when it reaches maturity, is deposited in a shady place near water where the soil is loose and moderately dry. The larva creeps into the loose earth, its skin becomes dark in colour, and in a short time it enters on the pupal stage which lasts from four to six weeks. The pupae are brown and barrel-shaped, eight to ten being produced by the female fly in its short life of three or four months. From the pupa the perfect insect emerges.

Fly Areas.

G. Palpalis is chiefly found close to water-courses where the banks are covered with sufficient vegetation to afford it shade. On Lake Tanganyika reeds give the necessary protection. On the Gold Coast it is found on the open beach in the loose sandy soil. It is not found in papyrus swamps.

Habits.

It is rarely found more than thirty yards from

water unless it has followed men or animals. It seldom bites except when the sun is up and the air still, and, as a rule, not through clothes. It does not occur at a greater altitude than 4,000 feet, and is seldom seen at such a high level. The prevalence of the fly is influenced by the seasons; in the wet season they become numerous; in the dry they may entirely disappear.

To avoid being bitten one must know its habits and be able to recognise the fly. Dr. Graham has suggested the following points which facilitate its identification on the wing.

"(1) It appears on the main roads as soon as there is sunshine, between 10 a.m. and 11 a.m. in the wet season, and earlier in the dry.

(2) It keeps near the ground.

(3) It's habit of flight is short, rapid, and furtive. Rising slightly from the ground as one advances, it flys forward a short distance keeping near the ground and is suddenly lost to view as it alights upon a small stone or projecting root on the roadway. And this it repeats each time it is disturbed until alarmed, when it takes cover in the vegetation by the side of the path.

(4) On being disturbed in a bush-path it usually flies swiftly several times past the intruder making a buzzing noise which is easily recognised.

(5) It alights by preference on the feet or legs of the victim, more rarely upon the back or arms.

(6) When driven off it disappears, settling upon the ground or under a leaf in the immediate vicinity and returns to the attack in a few minutes.

(7) It is alert and keeps out of view, changing its position from one side of the leg to the other when observed, but when it begins to draw blood it can be readily killed or caught.

(8) It attacks until sunset. It disappears after darkness sets in and is not attracted by artificial light."

There are certain places where one is especially liable to be bitten and great vigilance should be exercised in the following circumstances :

(1) Ina railway carriage. The fly usually approaches a train while it is standing in a station. It perches first upon the window sill, but soon seeks the floor or seat. Tabanidae on the contrary usually alight upon the walls or ceiling in sight. Women are the first to suffer as the fly creeps under the skirt and bites through the thin stockings. Therefore puttees or gaiters should be worn.

(2) In the tent - under the flaps, bed or other concealed position.

(3) In a ferry boat.

(4) At cross-roads, as here natives sit to rest and talk.

(5) At fords. Here natives sit before or after crossing.

(6) In narrow paths leading to a native village.

(7) Under the shade trees in a native village.

(8) In the open courtyard of native huts when cooking is in progress.

(9) In the path to the village water-supply.

(10) When passing a troop of native carriers in the morning. Some of the flies accompanying the troop leave it and attach themselves to the new party.

Thus a knowledge of the habits and identification of the fly will enable one to avoid being bitten if sufficient watchfulness is exercised.

When a fly is caught and an attempt is made to identify it, too much attention should not be paid to the mere crossing of the wings. This characteristic is common to many other flies and is not distinctive of the tsetse unless accompanied by a long proboscis, ensheathed in palpi, projecting in front of the head almost in the line of the body. Neglect of this precaution will cause it to be mistaken for the common mud fly, Ephydridae, or Stratiomyidae, a fly found upon leaves, etc.

Recent work on transmission has now made it

certain, as I will show in that section, that palpalis is not the only species of tsetse which can act as a carrier. In Northern Rhodesia and Nyasaland, which we know are outside the area of distribution of palpalis, Glossina Morsitans has been shown to be the transmitting agent of T. Rhodiense.

Kinghorn and Yorke in their recent work on the Luangwa Commission have brought forward striking proof of this and have come to the conclusion that G. Morsitans is the sole transmitting agent in these areas.

(3) The Tyypanosome.

1. T. Gambiense.

2. T. Rhodiense.

1. T. Gambiense is a fusiform mass of protoplasm which, like other trypanosomes, at one end passes into a pointed flagellum. It is 17 u to 28 u long by 1.4 to 2 u wide. Forms undergoing division are a little longer and considerably wider (2.5 u to 3 u) than the ordinary forms. The free flagellum is often one third or a quarter of the whole length; but sometimes the protoplasm of the body is continued along the whole length of the flagellum.

The minute structure is best stained by Leighman or Giemsa or other Romanowsky modification. In preparations stained by this method the protoplasm stains blue. Two bodies are always present in the

protoplasm. Near the middle there is an oval granular body staining purple - the tropho-nucleus or macronucleus - and towards the posterior end is a minute intensely stained purple granule known as the micronucleus or centrosome. The posterior end of the body varies in shape; sometimes it is rounded, sometimes it is pointed. These variations in the appearance of the posterior extremity are undoubtedly dependent upon the changes in shape which the parasite undergoes during its migrations. The parasite contracts and elongates alternately, so that the rapid drying of the blood fixes the parasite in one or other of these phases.

When the posterior end is drawn out and pointed the centrosome is further removed from the tip than when it is contracted and rounded off, so that the distance of the centrosome from the tip is a variable quantity and cannot be used as an aid to diagnosis.

Close to or around the centrosome there is often seen a clear vacuole, to which Castellani attributed great importance; and he looked on the position of this vacuole in relation to the centrosome as the principal differentiating feature between T. Ugandsuse and T. Gambiense. These are, however, artificial distinctions and the presence of a vacuole is now regarded as an artefact. In well fixed blood films vacuoles are not seen. They are most marked in badly

fixed preparations made from the cerebro-spinal fluid or from the blood of very anaemic individuals.

From the centrosome or its neighbourhood there arises the undulatory membrane. This is narrow, has a sharp undulating free margin, and surmounts the protoplasm of the organism like a cock's comb; it narrows towards the anterior end where it passes into the flagellum. Motion, as in the other trypanosomata, is chiefly affected by the undulations of this membrane and of the flagellum. The latter is continuous with the protoplasm of the body; it stains uniformly except at the free edge which has the reddish hue of chromatin.

The protoplasm often contains chromatin granules which are often remarkable for their size and number.

As has already been mentioned, the trypanosomes seen in a blood film may differ morphologically in appearance. Some are long and slender, stain well, and have an elongated nucleus and a long flagellum; others are broader and shorter, with a clear plain-staining cytoplasm containing many granules, with short flagellum, and well developed undulating membrane. These two types are regarded by Prowazek, Minchin, Zuthe, Nocht and Mayer, and others as being in all probability sexual forms, the slender parasites being looked on as the male and the broad as the female. In addi-

tion to these, there are the ordinary or indifferent forms.

The organism divides longitudinally in the usual manner; the centrosome and nucleus divide, then the flagellum and finally the protoplasm shows signs of division. The trypanosomes often occur in pairs, with their posterior ends overlapping to a considerable extent, and may thus give rise to the appearance of a trypanosome undergoing division. The presence of flagella at both extremities, however, prevents one from falling into this error.

As a rule the trypanosomes are scanty, or very scanty, in the blood of man, and the examination of the blood in the ordinary way is not sufficient to show the presence of the parasite, repeated centrifuging being necessary. This and other methods of showing the presence of the trypanosome, I will, however, descrive in detail in the section dealing with the Diagnosis of the disease.

2. T. Rhodiense.

This has now been definitely shown to belong to a distinct species and we are indebted to Kinghorn and Yorke for their valuable work on this subject.

The most striking morphological distinction between the T. Rhodiense and T. Gambiense is the posterior displacement of the macronucleus in the former. In every true case of T. Rhodiense is this observed.

Forms in which the macronucleus is actually posterior to the blepharoplast or centrosome are of extremely rare occurrence, whereas forms in which the macronucleus was displaced within the posterior quarter or fifth of the body, so that it came to lie in contiguity to the blepharoplast, were very common. This displacement constitutes, in Kinghorn and Yorke's opinion, the chief morphological peculiarity of the species. The time taken to complete its cycle in the fly is extremely short averaging about 13 days as compared to 17 days in the case of T. Gambiense.

The results of inoculation of various animals, however, form its most striking difference from T. Gambiense. T. Rhodiense is clearly distinguished by its greater pathogenicity for the majority of animal species. Whereas in rats and mice trypanosome Gambiense has a very variable virulence, some animals being refractory and others contracting a very light infection. Trypanosome Rhodiense invariably kills them. In the guinea-pig, dog, and macacus monkeys, the duration of T. Rhodiense infection is shorter than that of T. Gambiense. In sheep and goats the differences in the evolution, symptomatology and gravity of the two infections are quite remarkable. Whereas T. Gambiense infection in these animals gives rise to no symptoms except fever, often missed unless the temperature is

taken, and usually ends in recovery, T. Rhodiense infection leads to an acute disease with high fever, oedema and keratitis, and death is invariably the end after a relatively short duration.

(4) Mode of Transmission.

In 1909 the French Commission put forward the view that biting insects other than tsetse, for example, mosquitoes, could spread sleeping sickness. No case in which this has occurred has been put on record but an important piece of negative evidence has, however, come to hand. The Islands or Principe and San Thomé lie a few miles apart in the Gulf of Guinea. They have a similar population, similar vegetation, the same climate. Principe contains tsetse flies, San Thomé does not. On Principe there is much sleeping sickness; of nearly 2,000 persons examined by the Portugese Commission 23·5 per cent. were found to be infected. On San Thome the Commission was unable to find a single case; and this was not due to want of opportunity for cases of sleeping sickness are frequently imported into each island. On San Thome moreover, they found all the blood-sucking insects which they had seen on Principe, with one exception, the Genus Glossina. This is to my mind presumptive evidence that the transmission of sleeping sickness occurs only in the presence of tsetse flies.

Until quite recently it appeared that we could still adhere to the belief that among the tsetses the species palpalis was the only carrier, and no one could point to an undoubted case of infection in which the transmitting agent was other than palpalis. Unfortunately we now know that sleeping sickness is spreading in Northern Rhodesia and Nyasaland, and we are certain that many of these areas are outside the area of distribution of Glossina palpalis but within that of morsitans. Klein has shown that Glossina morsitans is not hospitable to T. Gambiense and Kinghorn and Yorke have done much to elucide the problem by their valuable work on T. Rhodiense. We must now look on both palpalis and morsitans as carriers, but of different species of Trypanosome,i.e. the T. Gambiense and T. Rhodiense.

Klein opened a new road to investigators by showing that tsetse flies could, after a latent interval, be permanently infected with pathogenic trypanosomes. That such late development istthe rule in insects which transmits trypanosomes from mammal to mammal seems probable. Bouffard has demonstrated it for Trypanosoma Cazalboui and Glossina palpalis and tachnichoides; Bruce and his colleagues for T. Vivax and Gl. Palpalis; and Minchin and Thompson for T. Lewesi and the rat flea. Moreover, there is little evidence that direct transmission ever occurs. Bruce

and his co-workers experimented to find out the part played by direct or mechanical transmission. A cage containing laboratory-bred flies (Gl. Palpalis) was placed for some little time on an animal infected with T. Gambiense, then suddenly transferred to a healthy animal, and so backwards and forwards for ten minutes. In this way a goat and a monkey were infected from a monkey. When there was an interval of half an hour to forty-eight hours between the feeds not a single case of infection occurred. The results make it fairly certain, as Sir David Bruce points out, that the successes of the former sleeping sickness Commission, after 24 and 48 hours as it was supposed, were due to late development, the same flies having been used throughout. The conclusion is that mechanical transmission plays a small part, if any, in the spread of sleeping sickness. The same observer found that infectivity did not appear till about thirty-two days after the fly had fed and continued until at least seventy five days. Klein, too, obtained no evidence of direct transmission after an interval. He fed 1,910 tsetse flies on infected animals and eighteen to twenty hours later on healthy animals; there was no case of direct transmission.

When the development in the fly was discovered, the non-infective interval appeared to be a definite

fixed period. A long series of experiments by the sleeping sickness Commission seems to demonstrate that it may be very variable. Both wild and bred flies were used. The experimental animals were monkeys. In seven positive experiments with wild flies the shortest time which elapsed before a fly became infective was eighteen days, the longest forty-five, and the average thirty-two. With laboratory-bred flies, which are more difficult to infect, these periods are longer, the average being thirty-five days. In neither series was it found easy to infect the flies and in all cases the flies were examined at the end of the experiment for flagellates. As a result, it was concluded that T. Gambiense multiplies in the gut of about one in every twenty Glossina palpalis which have fed on an infected animal. This is the same proportion as was obtained by Klein. There is no reason to suppose that it is ever as high as this in nature; for from the recorded transmission experiments it has been deduced that not more than 2·5 per thousand flies are infected in natural conditions, 11 per thousand being the highest in any experiment. Why the proportion of infectible flies is so small can only at present be explained by the hypothesis of Bruce, that it is a case of the survival of the fittest; in a few flies a few hardy parasites not only survive, but reproduce their kind, and in course of time become capable of infecting

a new vertebrate.

There is evidence that T. Gambiense may multiply in the fly without becoming infective. In six cases the Commission examined flies at the end of the experiment and found that they contained flagellates. These must have been T. Gambiense, but they had not transmitted the disease in biting, nor did their introduction into healthy monkeys produce infection. In other instances the injection of infected flies or of fluid from the alimentary canal into susceptible monkeys produced sleeping sickness. The infected flies remained capable of transmitting the disease till their death, which in the longest-lived was on the seventy-fifth day.

We are still in the dark as to what goes on in the insect during the non-infective period. The fact that the trypanosomes in the alimentary canal are infective goes to show that there is no invasion of the body fluids and afterwards of the salivary glands, such as Chigas observed in the case of Schizotrypanum. There is no evidence that T. Gambiense is infective to the tsetse fly at one stage of its life history more than another and there seems to be no such thing as hereditary transmission in the fly of those trypanosomes which are pathogenic to mammals.

Trypanosoma Rhodiense.

The Luangwa Commission issued their report in 1910. In their experiments they used laboratory-bred and wild Glossina morsitans, and in both series they were successful. They also ascertained that trypanosomes, corresponding in every particular to the human variety were being transmitted by this tsetse fly in nature. The conclusion that the trypanosome was the human one was based both on the morphology and the animal reactions.

The most striking feature was the shortness of the time taken by the trypanosomes in completing their cycle in the fly: 13, 15, and 11 days as compared to 32 days in the case of T. Gambiense. The infective flies did not require to feed more than a single time in order to infect any animal, nor had the flies to feed more than once to become infected themselves.

The conclusions of the Commission are as follows :

(1) The human trypanosome, in the Luangwa valley, is transmitted by Gl. Morsitans.

(2) Approximately 5 per cent. (4.76) of the flies may become permanently affected and capable of transmission.

(3) The non-infective period, between the infective feed of the flies and the date on which they themselves become infective, is approximately 14 days.

(4) An infective fly retains the power of transmitting the disease during its life and is infective at each meal.

(5) Mechanical transmission does not occur if 24 hours have elapsed since the infective meal.

(6) Gl. Morsitans, in nature, has been found to transmit the human trypanosome.

(7) Certain species of buck have been found to be infected with the human trypanosome.

Perhaps the most striking discovery in the period under review was that of Bruce in Uganda, that the flies caught on the shore of the Victoria Nyanza, cleared of inhabitants for some two years, were able to infect monkeys with sleeping sickness. Various causes of this continued infectivity have been suggested and Bruce in 1910 writes, "The opinion is growing in my mind that it is more than probable that the wild game on the lake shore will be found to act as a reservoir." This has not been definitely proved.

Bruce, Hamerton and Bateman showed that numerous species of buck and antelope could be readily infected, and that they could then transmit the infection; and Kinghorn and Yorke, in their recent paper, have placed the question beyond debate by finding in the Luangwa valley that these animals harbour a human parasite morphologically and by its animal reactions, identical with T. Rhodiense. Duke is the only observer

who has found T. Gambiense in wild game in spite of numerous and exhaustive experiments; and the present conclusion is that human trypanosomes are much rarer in antelope in Uganda than in Rhodesia.

Now that it is proved that wild animals form a reservoir of the sleeping sickness virus, it is plain that all places where such animals and the glossina coexist will be uninhabitable by man till the circle is broken by the extermination of either the vertebrate or invertebrate host. The "tsetse-fly and big game" has now become a burning question in human as well as animal pathology.

(5) Predisposing Causes.

Age appears to have no marked influence upon the disease. The disease has been seen in many infants of eighteen months to two years, and these children had become infected a considerable time previously. The two sexes are attacked in the same proportion. The influences of occupation and social position are very marked. The majority of cases are seen amongst the poorer classes. Native chiefs and persons belonging to the upper classes are attacked in a much smaller proportionthan the poorer negroes who work in the fields all day. Europeans and Asiatics are not exempt.

The seasons exert no influence on the course

of the disease.

Wars and famines have favoured the spread of sleeping sickness and, though naturally endemic, it may assume an epidemic form. The Soudanese soldiers in 1888 contributed to the rapid spread throughout Uganda; and along the Ivory Coast, before its invasion by Samouri, the disease was much less common than it is at the present day.

Like all diseases which are propagated by a special insect, the disease is infectious only where the insects capable of propagating it occur. In districts where the insect is absent the introduction of infected individuals is followed by no serious results.

Pathology.

On considering the disease from the standpoint of pathological anatomy there is little to be said. As Mott described, the most striking feature is the presence of a Chronic Meningo-Encephalitis and Meningo-Myelitis. The pia-arachnoid is sometimes opaque and slightly thickened and may be adherent to the brain, and its vessels usually show some congestion. The sub-arachnoid fluid is sometimes in excess and occasionally may even be purulent. The membranes of the spinal cord show similar changes.

On cutting into the brain the only morbid conditions present as a rule are excess of fluid and dilatation of the lateral ventricles.

The other chief feature is the presence of lymphatic glands in the body, but otherwise there is nothing special to note. The change in the glands is inflammatory in nature, and terminates in fibrosis. Trypanosomes are always very scanty in sections of glands, so that the chronic irritation which they set up in the lymphatic glands generally, and later in the lymphatics of the brain and spinal cord, is probably not due to their mere mechanical presence, but to a chemical toxin.

The posterior spinal ganglia always show chronic inflammatory changes.

With regard to the microscopic features, the chief change, according to Mott, is a proliferation and overgrowth of the neuroglia cells, especially those which are related to the sub-arachnoid space and the perivascular lymph spaces, with accumulation and probably proliferation of lymphocytes in the meshwork. He further points out that the changes in the lymph glands are of a similar nature and resemble the infiltration of the perivascular lymphatics of the central nervous system. These changes are especially significant in view of the lymphocytosis present in the blood and which so often occurs in protozoal infections.

In the nervous structures there is comparatively little change, there being merely, according to Mott, some atrophy of the dendrons of the nerve cells, a diminution of Nissl's granules, and an excentricity of the nucleus.

On the whole the changes found in the nervous system very closely resemble those found in syphilis, except that proliferative endarteritis is absent.

The other tissues, e.g. the heart, liver, spleen, intestines and testicles, show, though in a far less degree, an infiltration and accumulation of lymphocytes in the lymphatics. Enlargement of the spleen and liver is frequently seen.

Symptoms.

In a disease so long known as sleeping sickness, it is not to be expected that much would remain unrecorded in the semeiology of the disease. An analysis of the symptoms in fifty published cases of sleeping sickness in Europeans has lately been published in the "sleeping sickness Bureau Bulletin." According to this the most frequently recorded are, in descending order : fever, erythema, loss of strength, increased frequency of heart beat, somnolence, oedema, large spleen, anaemia, headache, and epileptiform convulsions. The relative position of symptoms in the scale depends to some extent on the proportion of advanced to early cases which did not progress. Thus if many of the cases tabulated are advanced, such symptoms as somnolence and convulsions will rank high. Other common symptoms are indefinite pains about the body, the evening temperature may be elevated several degrees, and the pulse tends to become soft and rapid, and the superficial glands of the body are enlarged.

While there is a general likeness in the symptoms in the recorded cases, one hears now and again of a case in which none of the characteristic signs are present, e.g., one recorded by Dr. Hearsey, in which there was only severe headache and oscillating temperature. Death occurred in the sixth week with the disease undiagnosed. Severe headache seems almost

characteristic of severe T. Gambiense infection in man.

Thiroux has described skin lesions which he believes to be those of Trypanosomiasis. These consist of papules, scattered or in patches, and sometimes ulcerated.

Morax has described a case of choiroido-retinitis which he believes was the result of trypanosome infection, and he was able to exclude syphilis as the Wassermann reaction was negative. The choiroido-retinitis came on at the same time as cyclitis which is a recognised symptom in sleeping sickness.

Van Someren and Kopke believe that lesions of the optic nerve may occur apart from the treatment by organic arsenals. Such changes do not influence the vision but would certainly predispose to blindness a patient treated by organic arsenals. Spielmeyer, it may be noted, found degenerative changes in the optic nerve and tract of dogs infected with Nagana.

Diagnosis.

Little of importance has been published on this subject during the past year. It is now recognised that gland palpation, combined with gland puncture, employed in the period when occasional fever is the only symptom, and while the patient is able to pursue his ordinary vocation, will not discover even a large proportion of the infected. Wherever serious attempts are made to detect cases of trypanosomiasis the blood is examined as well as the glands. Dr. Kinghorn , formerly a strenuous advocate of gland palpation, found five cases by the blood method among 119 natives who had previously been examined by the gland method with a negative result. It would appear that in the natives on the Gold Coast glandular enlargement may not be met with in a fair proportion of those infected, and that, consequently, the examination of blood preparations is of equal importance to that of the glands.

In 45 cases of trypanosomiasis in whites the parasites were found in no less than thirty-two instances first in the blood, and only in six by gland puncture. This was doubtless because in many of the thirty-two the glands were not explored, but it serves to illustrate the value of blood examinations in this disease. The drawback of the blood method is that in many in-

stances the trypanosomes are scarce, but with repeated centrifugalisation with sodium citrate and good technique this difficulty may be overcome. The discovery that there is a trypanosome tide once in every seven days suggests that it is advisable, in cases in which the thick-film method is not applicable, to repeat blood examination daily for at least a week so as to include the flood. Whether the thick film or the more usual technique of blood examination should be used depends entirely on the skill of the observer; to the inexperienced the former presents difficulties which render his observations more or less valueless.

The cerebro-spinal fluid should be examined by lumbar puncture. The fluid is centrifuged and the deposit examined. The trypanosomes are never very numerous and, as a rule, fix and stain badly. The fluid is rarely as limpid as normal.

More observations have lately been made on auto-agglutination of the red blood corpuscles in cases of trypanosomiasis. Todd found that it was at its maximum just after trypanosomes had been numerous in the blood; and he published a table giving the proportion of positive results in 1,406 cases examined in the Congo State. It shows that, while auto-agglutination may be absent when trypanosomes are present, of cases in which the corpuscles clumped a large proportion harboured trypanosomes. It remained, there-

fore, true that well marked clumping of the red corpuscles, ina person who has resided in an infected area, is a phenomenon which should lead the observer to make repeated search for trypanosomes. Leach and White have come to the same conclusions.

A study of the published leucocytes counts, especially in Europeans, makes it certain that lymphocytosis nearly always occurs, and that the increase is chiefly at the expense of the polymorphs. An average of counts from forty-six patients is as follows:-

Polynuclears.	40.75.
Large Mononuclears.	14.13.
Lymphocytes.	38.31.
Eosinophiles.	6.81.

If in a doubtful case this altered relation were demonstrated it would go to support the diagnosis of trypanosomiasis, but it must be remarked that such an altered relation has been recorded, apart from disease, in whites long resident in the Tropics.

Laveran and Nattan-Larrier have been experimenting to see if reliance can be placed on the reactions of trypanolysis and attachment for diagnosis. The results were inconstant. They have come to the conclusion that trypanolysis is of little value and that the attachment reaction is, if possible, even less satisfactory.

Objective signs are, of course, of great value those of most importance being pyrexia, tachycardia, and rapid changes in the frequency of the pulse (without relation to temperature), puffiness of the face, tremor of the tongue, general shakiness and vacant expression.

Malaria and filariasis are the diseases most likely to be mistaken for it, but the periodicity of the pyrexia, the effects of quinine and the results of blood examination should prevent mistakes. The fact that these diseases frequently coexist should not be overlooked.

Treatment.

In the last twelve months no new trypanocide has come into use. We have learned little more as to the use of the arsanilates such as atoxyl and soamin. Arsenophrenglycin and Arsenobenzol (606) promised well but have been disappointing.

Arsenophenylglycin.

Von Raven has obtained the most favourable results with this drug, results which are largely due to his following the method insisted on by Ehrlich by giving one or two large doses and no more. In a series of cases no relapse had occurred eleven months later.

Unfortunately the patients are still liable to re-infection so that if trypanosomes reappear we shall be unable to say whether we have to do with a relapse or a re-infection. He gives the following advice as to the employment of the drug; to exclude advanced cases; to give a single injection, or two on adjacent days, of at least 0.8 to 1.0 gm; if relapse occurs within six weeks to change the treatment. Ehrlich emphasises the importance of getting in the trypanocide when parasites are numerous in the blood.

Tryparosan and Aniline Antimonyl Tartrate.

These drugs introduced by Ehrlich promise to be of use.

Tryparosan is best given in 4 or 5 gm. doses by the mouth. It is not suggested that this dye can replace the more powerful trypanocides but it should prove a useful adjunct.

Aniline antimonium tartrate is best given intravenously in association with atoxyl. It is of doubtful value.

Arsenobenzol is strongly advocated by Ehrlich but the lack of technique shown by observers up to date render their observations worthless. With the necessary apparatus in scientific hands the results should be most promising.

As to the older methods, Medical Officers in charge of camps in Uganda favour a short course of atoxyl or soamin, followedbby a longer one of orpiment, and Van Someren has had good temporary results from the simultaneous use of soamin and perchloride of mercury. Martin and Darre advocate atoxyl and orpiment in mild infections, atoxyl and antimony in the more severe and for Europeans.

None of the trypanocides at present in use can be relied on to banish the parasites from the cerebrospinal fluid.

The action of the arsanilates on the eye has been studied by Igersheimer. He has come to the conclusion that atoxyl has a selective action on the nerve cells of the eye and that the point of attack may be

central or peripheral, or may lie in the course of the optic nerve. A strong affinity existed in the bulb for the atoxyl molecule but not for inorganic arsenic. atoxyl amblyopia, in fact, seems to depend on neither the arsenic nor aniline constituent. Many cases of blindness have also been reported after the use of soamin and arsacetin.

The course of the disease is said to be favourably influenced by an attack of pneumonia; and Thiroux who has also reported apparent cures after quite inadequate treatment, has suggested that this is due to the presence of antibodies.

Time has only emphasised the inefficacy of any form of treatment; in all but the earliest cases life may be prolonged but recovery can seldom be hoped for. For the ultimate conquest of the disease it is evident we must depend on preventitive measures.

The broad principles of prophylaxis have been known since the discovery that Glossina palpalis transmits the disease. These consist either in the removal of natives, infected or not infected, from the areas haunted by the fly, or in the destruction or banishment of the fly itself. Obviously the latter is the true prophylaxis, and now it is proved that wild animals harbour the virus, it is to measures directed against the fly that we must look more and more.

The only certain way at present known of gett-

ing rid of tsetse flies in clearing, i.e. removing the vegetation which shelters them and affords opportunities for breeding. Removal of native villages and the drugging of their inhabitants is at best palliative.

As a rough guide the ground should be cleared for 100 yards around watering places or landings for canoes. As a rule the flies will disappear when such clearings are made (except those which are conveyed by boats). If, however, this measure fails it is necessary to search for the pupae. These are easily collected in large numbers: On one occasion 7,000 were brought in by fly ~~fogs~~ boys.

To prevent rank vegetation springing up again it is necessary to plant some crop. Good results have been got by the cultivation of lemon and Bermuda grasses.

Trapping of flies may be tried, by means of natives wearing black cloth smeared with bird-lime or other sticky substance, but mainland flies are limed with difficulty. Various native dyes and volatile oils, such as eucalyptus, are distasteful to the fly and are useful tsetse fuges.

Camping at the edge of a fly area should be avoided; a safe distance will seldom be less than half a mile. Fords infested with flies should be crossed if possible in the early morning or after sunset.

Native huts or European houses should be moved

out of the fly range, at least half a mile from the water, and if possible on to higher ground.

Infected persons should be moved well out of the fly area and a spot should be chosen free from all tsetses. One infected person within a fly area may cause the infection and destruction of a whole village.

If European houses must remain within the fly range they should be protected by wire gauze. Steamers which ply on fly-infected rivers should be provided with wire-gauze cages into which Europeans can retire.

To go about with bare arms and legs is to invite infection. Women must wear puttees or leggings as stockings are an insufficient defence.

Summary and Conclusions.

Now that it is known that sleeping sickness is being diffused in Rhodesia by a tsetse fly other than palpalis, why has the disease not spread in East Africa by the agency of Glossina pallipides and fasca, or on the high ground in the Katanga by that of Glossina Morsitans? Bagshawe suggests as a provisional hypothesis that it is because in these uplands the air is too cool and too dry to admit of the parasite's completing its development in the body of the fly. This is a feasible explanation and there is abundant analogy in nature. It is well known that certain ticks harmless on high ground become bearers of disease when brought into valleys. In malarial mosquitoes development takes place more and more slowly as the temperature falls, till at a certain point it ceases to be completed. The same reason may account for the disappearance of yellow fever with the advent of cold weather. On Lake Nyasa, on the other hand, and in the Luangwa valley the altitude is low, the air is warmer as well as moister than at higher levels; and, as has been shown, these flies are taking the role played by palpalis in other parts of Tropical Africa.

A point worthy of notice in the Rhodesian disease, is the slow rate of spread even under the most favourable conditions, whereas the disease should spread rapidly in places where a carrier is so abundant and

blood-thirsty. Recent experimental evidence, however, explains this. In the laboratory only 4.76 per cent. of morsitans became permanently infected and the infection rate in nature probably does not reach such a high level. Again, sleeping sickness appears to have spread very slowly in some areas where palpalis was abundant, and in every case the disease has been long in establishing a footing. It is quite conceivable that the rate of spread in the case of Gl. Morsitans being an active transmitter, as opposed to a mechanical one, would be much slower.

It is to be hoped that all the causes of these extra-palpalis infections will soon be demonstrated. Investigations by skilled and well-equipped workers should be promptly undertaken; for the mere suspicion that palpalis may owe its bad reputation to its habits and habitat rather than to any differences which may exist between it and the other species would cause a feeling of insecurity in all parts of South Africa where tsetse flies are found. In Northern Nigeria tachinoides exists in large numbers under precisely the same conditions as palplais elsewhere, but up to the present, as far as we know, these parts are free from sleeping sickness. The fact, however, that not till seven years after the discovery of the association between palpalis and sleeping sickness has undoubted evidence of transmission in the absence of

palpalis come to light, suggests that only rarely are the necessary conditions fulfilled in the case of morsitans, and the rest, and that we are still justified in regarding Glossina palpalis as the transmitter.

Other promising lines for research might be suggested. What is the life-cycle in the insect during the non-infective period? No evidence has been obtained that germinal transmission is possible. Though no cases of infection by sexual coitus have recently been reported, experimental work suggests that it may occur. Martin and Ringenbach found that by allowing blood containing T. Gambiense to run into the vagina they should infect guinea-pigs.

Not long ago it was doubted whether persons once infected ever recovered. We have now many years' experience of the disease in Europeans, and a study of the published cases leads to the belief that recoveries almost certainly occur, and if treated early may not be infrequent. At present, unfortunately, we have no certain test of cure. Even suspeptible animals may fail to be infected when trypanosomes are scarce. I would strongly emphasise the value of research on this point. It would give us the correct valuation of present methods of treatment. At present it is impossible to say what share chemo-therapy has had in recovered cases; it is possible that the infections were mild and that they would have recovered without the

use of drugs.

With regard to natives the records from the Uganda camps show that 90 per cent. were dead at the end of the third year and less than one per cent. were "thought to be probably cured." It is not easy to see why so very few natives of Uganda have made an apparent recovery and throws further doubt on the value of treatment by drugs. Of forty-two Europeans infected, fifteen survived more than three years after their disease was diagnosed, a very different percentage ~~to~~ from that in Uganda. The difference suggests that, if we could supply the natives with the food and improved conditions of the white man, we should benefit them more than by the administration of atoxyl.

In conclusion I would again point out that time has only emphasised the inefficacy of treatment by drugs. No reliance can be placed on any trypanocide at present in use and we must depend on prophylaxis to keep this tropical scourge in check. The only true prophylaxis is the destruction of the fly itself. Clearing should be carried out on a large scale by an organised service under the direction of administrative officers of experience. Where it is possible, the cleared land should be brought under cultivation or sown with grasses which do not encourage the breeding of the fly. The cleared areas should always be kept under surveillance and new vegetation prevented from

springing up again.

Recent work on drug prophylaxis suggests that some efficient preventive may be found inthat direction.

Morgeuroth finds that when mice are fed with quinine a few days before inoculation with T. Brucei, no infection occurs. This is surely a promising line of research.

Bibliography.

General.

Laveran and Mesnil. "Trypanosomes et Trypanosomiasis."

Minchin in Allbutt's "System of Clinical Medicine," 2nd edition.

Schandin, Arbeit. a. d. Kaiserl. Gesundheitsamte, XX. 387.

Mense, "Handuch der Tropenkrankheiten," Leipzig, 1906.

Novy and MacNeal, Journ. Inf. Diseases, ii. 256.

Leishmann, Journ. Hyg. IV. 434.

Minchin and Thompson, Proc. Roy. Soc. London, B. lxxxii. 273.

Chagas, Bull. de l'inst. Pasteur (1910) viii. 373.

Sleeping Sickness.

Mott, Reports of the Sleeping Sickness Commission, pt. vii. No. 15, London, Bale Sons and Dannielsson.

Dutton and Todd, B.M.J. (1903), i. 304.

Dutton and Todd, Thompson and Yates, Lab. Rep. v. pt. II. I; V. pt. II. 97. Dutton and Todd and Christy, ibid. VI. pt. I. p. 1.

Manson and Daniells, ibid. (1903), i. 1249.

Idem, ibid, II. 1461.

Low and Mott, ibid. 1. 1000.

Bettencourt, Kopke, Resende, and Mendes, ibid. 1. 908.

Castellani, Reports of Sleeping Sickness Commission, No. I. i. London.

Bruce and Nabarro, ibid. No. 1. 2. 11.

Bruce, Nabarro and Greig, ibid. No. IV. 8. 3.

Greig and Grey, ibid. (1905), No. 6, 2. 3.

Leishmann, Journ. Hyg. IV. 434.

Minchin, Grey and Tulloch, Rep. of Sleeping Sickness Commission, No. 8, 21, 122, London. H. M. Stationery Office (1907).

Manson, B.M.J. (1903) II. 1249, 1461.; B.M.J. (1903) II. 637, i. 1904, II. 365.

Thomas, Thompson, Yates, Lab. Rep. VI. pt. II. I.

Kleine, Deutsche Med. Woch. (1909) pp. 469, 924, 1257, 1956.

Bruce, Hamerton, Bateman and Mackie (Sleeping Sickness Commission to Roy. Soc. 1909) Proc. Roy. Soc. London. B. 81. 405; ibid. 82, pp. 56, 63, 256, 268, 480, 485, 498.

Sleeping Sickness Bureau Bulletin, Vols. 1, 2, 3, 4.

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